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14. ABSTRACT
Breast cancer is a common disease in women but the causes are still largely unknown. There is considerable evidence to suggest that genetic factors play an important role in causing breast cancer, but the genes involved in the majority of breast cancers are currently unknown. Our aim is to identify genetic factors that increase the chance of breast cancer occurring. We have collected clinical information and samples from over 1500 breast cancer families. We will compare the frequency of genetic factors in these cases with control women without breast cancer. Initially we will analyze genes that we suspect may have a role in breast cancer. However over the course of the study we plan to use new technologies to analyze every gene (~30,000 genetic variants). If we find any variants that are more frequent in breast cancer cases than controls, it suggests that they may be involved in causing breast cancer. We will evaluate these variants in further cases and controls to prove an association with breast cancer and to define the risk and outcomes of carrying the genetic variant(s).

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INTRODUCTION

Breast cancer is a common disease in women but the causes are still largely unknown. There is considerable evidence to suggest that genetic factors play an important role in causing breast cancer. In the last decade considerable progress has been made and two major breast cancer genes, *BRCA1* and *BRCA2*, have been identified (Rahman and Stratton, 1998). These genes carry a high risk of breast cancer but only account for a very small proportion of breast cancer families. Weaker genes are likely to be involved in the majority of familial breast cancers and some breast cancer cases without a family history of the disease, but few have been identified (Antoniou and Easton, 2003; Meijers-Heijboer et al. 2002).

Our aim is to identify and characterize the genetic factors that increase the chance of breast cancer occurring. We have collected clinical information and samples from over 1500 breast cancer families. We are first characterizing these for the known breast cancer genes, *BRCA1* and *BRCA2*, with particular emphasis on clarifying the contribution and nature of large rearrangements of these genes, which have recently been identified in some familial breast cancer pedigrees and which are not identifiable by gene sequencing. We will then proceed to try to identify new genes, by comparing the frequency of genetic factors in these cases with control women without breast cancer. Initially we will analyze genes that we suspect may have a role in breast cancer. However over the course of the study we plan to use new technologies to analyze every gene. If we find any variants that are more frequent in breast cancer cases than controls, it suggests that they may be involved in causing breast cancer. We will evaluate these variants in further cases and controls to prove an association with breast cancer and to define the risk and outcomes of carrying the genetic variant(s).

BODY

As part of the program of work we defined five tasks, two of which we aimed to start in the first year. Although the grant has been active for one year we needed to recruit four members of staff to undertake the work and this took four-six months. We have therefore only been working to full capacity for six months. Nevertheless we have made substantial progress towards our outlined aims. The progress towards the tasks is outlined in detail below.

Task 1: Evaluate the contribution of BRCA1 and BRCA2 exonic deletions and duplications to breast cancer susceptibility.

We have undertaken analyses for genomic exonic deletions and duplications of *BRCA1* and *BRCA2* in 856 familial breast cancer cases from separate pedigrees in which mutations of these genes have been excluded. We use a simple, cost-effective copy number analysis technique, multiplex ligation-dependent probe amplification (Schouten et al. 2002; Bunyan et al. 2004). This analysis has resulted in the identification of the following 27 abnormalities (Table 1). Each finding has been repeated in triplicate using fresh template. 19 abnormalities were identified in *BRCA1* and 8 in *BRCA2*. The overall frequency of large deletions / duplications in *BRCA1* and *BRCA2* in UK familial breast cancer is therefore ~3%.

Table 1. Exonic deletions and duplications identified in *BRCA1* and *BRCA2*

Abnormality	Gene	Number of families
Del exons 1-2	BRCA1	5
Del exon 1	BRCA1	1
Del exon 1-7	BRCA1	1
Del exon 1-23	BRCA1	1
Del exon 9-12	BRCA1	1
Dup exon 13	BRCA1	6
Del exon 14-20	BRCA1	2
Dup exon 21	BRCA1	1
Del exon 20-22	BRCA1	1
Del exon 1-2	BRCA2	2
Del exon 8-10	BRCA2	1
Del exon 14	BRCA2	1
Del exon 14-16	BRCA2	4

During the course of this experiment we have discovered that whilst MLPA analysis is simple to run and cheap (£8 per assay), a number of potential limitations require further investigation and evaluation.

1) MLPA analysis is much more sensitive to DNA quality than sequencing which can lead to both false positive and false negative results. Sub-optimal DNA quality is detectable as it results in probe variability for the whole assay. We are therefore defining the parameters of optimal and acceptable assays with a view to disregarding all results for which DNA

quality is below a pre-defined threshold. In such cases we would aim to collect a new sample for analysis.

2) False positive results can occur for single exon deletions if there is a polymorphism or mutation in one of the MLPA probe sequences for that exon. We therefore now sequence fragments containing all apparent single exon abnormalities.
3) Certain probes in the kits give variable results especially for exons 1 and 2. We have notified the kit suppliers who are evaluating new probes that may be able to be substituted or included in addition to the existing probes.
4) The frequency of *BRCA1* large rearrangements is greater than *BRCA2* rearrangements (19 compared with 7) despite the fact that *BRCA2* mutations make a larger contribution to familial breast cancer pedigrees than *BRCA1* mutations overall in our series (and in the UK generally). The reasons for this are unclear and merit further investigation. In the first instance we plan to extend our analyses to include another 1000 familial breast cancer pedigrees.

Task 2. Perform familial case-control analyses of non-synonymous coding single nucleotide polymorphisms (SNPs) in DNA repair genes in familial breast cancer cases, Months 1-36:

a) *Complete identification of coding SNPs by full gene screening of ~50 DNA repair genes in 96 non-BRCA1/2 familial breast cancer cases.*
b) *Analyse all non-synonymous coding SNPs identified in (a) in 500 additional non-BRCA1/2 familial breast cancer cases and 500 controls.*
c) *Analyse SNPs that show positive association with breast cancer in (b) in 10,000 unselected breast cancer cases and 10,000 controls.*

We have identified 114 non-synonymous coding single nucleotide polymorphisms (SNPs) in DNA repair genes through our sequencing of DNA repair genes in 92 *BRCA1/2* negative cases (of the 96 cases included in the original experiment, in two we identified covert *BRCA2*

abnormalities and two samples failed in multiple fragments and therefore we have excluded them). We have prepared an array that contains these SNPs ready to analyze in phase (b) of this task.

Initially we proposed to analyze 500 familial cases and 500 controls but stated that if the costs decreased we would analyze more samples as this would increase the power of the experiment. Fortunately, the costs of the analyses have decreased sufficiently that we are now planning to analyze 900 non-*BRCA1/2* familial breast cancer cases and 1000 controls. We anticipate that the genotyping results of phase (b) will be available for analysis within the next 12 months.

Task 3. Characterise the histopathology and immunohistochemistry of familial breast cancer. Months 12-36:

a) *Perform detailed pathological review and immunohistochemical analysis of at least 150 non-BRCA1/2 familial breast cancers.*
b) *Compare pathology and immunohistochemistry of non-BRCA1/2 familial cancers, BRCA1 cancers, BRCA2 cancers and unselected breast cancers.*
c) *Define pathological / immunohistochemical characteristics of non-BRCA1/2 cancers which may allow stratification into subgroups that facilitate identification of underlying susceptibility alleles.*

We have not started this task as yet.

Task 4. Perform genome-wide familial case-control analyses of non-synonymous coding SNPs, Months 12-48:

a) *Analyse ~30,000 non-synonymous coding SNPs (at least 1 from every gene) in 400 non-BRCA1/2 familial cases and 400 controls.*

a) *Evaluate top 5% (1500 SNPs) in 800 cases and 800 controls.*

We have made some progress towards this task, identifying 15,000 non-synonymous coding SNPs from ~ 50% of known genes. Unfortunately, the harvest of exonic SNPs by sequencing centres has been slower than anticipated and so all genes have not yet been sequenced, though this is likely to be completed within the next 18-24 months. In view of this we have decided to undertake this experiment in two phases and will analyze the 15,000 identified thus far and then analyze a second set of SNPs once all genes have been screened for SNPs. As the costs of analyzing SNPs have decreased this alteration in strategy will not increase the costs. In fact we will now be able to analyze 900 familial breast cancer cases and 1000 controls, greatly increasing the power of the experiment. We anticipate that the genotyping results of the 15,000 SNPs will be available for analysis within the next year.

Task 5. Identify low penetrance breast cancer susceptibility alleles, Months 36-60:

a) *Evaluate top 30-50 SNPs identified in Task 4 in 10,000 unselected breast cancer cases and 10,000 controls to identify which are truly associated with breast cancer and to determine the risks and phenotype in families and isolated breast cancer.*
b) *Evaluate novel breast cancer susceptibility alleles in BRCA1 / BRCA2 / CHEK2* 1100delC families to determine whether they modify or interact with these genes in breast cancer.*

This task has not been started as yet.

KEY RESEARCH ACCOMPLISHMENTS

1) We have completed copy number analyses of *BRCA1* and *BRCA2* in 856 familial breast cancer cases in whom BRCA1/2 intragenic mutations have been excluded.
2) We have identified 27 exonic deletions / duplications, 19 in *BRCA1* and 8 in *BRCA2*.
3) We have identified 114 non-syonymous coding SNPs in DNA repair genes in breast cancer cases and have prepared an array including these SNPs.
4) We have identified 15,000 non-synonymous coding SNPs though in silico data mining.

REPORTABLE OUTCOMES

None

CONCLUSION

The research program is proceeding on course. In our first year we have made substantial headway towards our stated aims, which we will consolidate and expand in the next year. There have been no major problems or alterations in strategy.

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APPENDICES

None

SUPPORTING DATA

None